EVALUATION OF PRO- AND ANTIOXIDANT EFFECTS OF MOLECULAR HYDROGEN ON EXPERIMENTAL CHRONIC HEART FAILURE INDUCED RATS

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Abstract. The world's biggest killer is ischemic heart disease, responsible for 16% of the world's total deaths. Since 2000, the largest increase in deaths has been for this disease, rising by more than 2 million to 8.9 million deaths in 2019. In recent years, many studies have shown that hydrogen has therapeutic and preventive effects in various human and animal disease models. In this study, we investigated the possible antioxidant effects of molecular hydrogen in erythrocytes and blood plasma in rats with the experimentally simulated chronic heart failure. We estimated the intensity of lipid peroxidation processes by the contents of diene and triene conjugates, Schiff bases, malonic dialdehyde, catalase activity. The results from this study suggest that inhalation of 2% molecular hydrogen leads to a decrease in pro-oxidant and an increase in antioxidant parameters. The results of this study provide the basic data for the mechanism research and application of molecular hydrogen in the future.

Keywords: molecular hydrogen, lipid peroxidation (LPO), malonic dialdehyde (MDA), catalase activity.

List of Abbreviations

CHF – Chronic heart failure
LPO – Lipid peroxidation
ROS – Reactive oxygen species
DC – contents of diene
TC – triene conjugates
SB – Schiff bases (SB)
PUFAs – polyunsaturated fatty acids
MDA – malonic dialdehyde

Introduction

Chronic heart failure (CHF) is one of the most common chronic diseases worldwide (Bowen et al., 2020). CHF is comparable to particularly dangerous infectious epidemic diseases in terms of the scale and speed of spread (GBD 2019 Diseases and Injuries Collaborators, 2020). The number of hospital admissions with decompensated heart failure has tripled over the past 15 years. Currently, it is an indisputable fact an important role in the pathogenesis of CHF belongs to the intensification of lipid peroxidation (LPO) processes mediated by immuno-inflammatory reactions with increased expression of pro-inflammatory cytokines (Gianazza et al., 2021). At the same time, there is still no effective and safe method of treatment and prevention of diseases accompanied by a violation of the processes of free radical oxidation (van der POL et al., 2019). A number of studies note the possibility of using molecular hydrogen as a universal antioxidant (Ohsawa et al., 2007; Ohta, 2011). The therapeutic effect of hydrogen has been proven for diseases whose pathogenesis is associated with the toxic effect of reactive oxygen species (ROS). It has been shown to be effective in ischemia / reperfusion injury of the brain (Ohsawa et al., 2007) and myocardial infarction (Yoshida et al., 2012). In this study, we investigated the possible antioxidant effects of molecular hydrogen in erythrocytes and blood plasma in rats with the experimentally simulated chronic heart failure. The mechanisms underlying the effects of hydrogen have also been investigated.

Materials and Methods

The experimental study was conducted on (n = 30) white male Wistar rats weighing 200 ± 20 g. Animals were maintained under standard conditions in individual boxes with a

natural 12-hour light-dark cycle, air humidity of 60% and temperature of 22 ± 2 °C, and were fed a normal diet.

All procedures and manipulations were conducted in accordance with the regulatory documents provided in the «Guide for the care and use of laboratory animals» and the requirements of the order of the Ministry of Health of the Russian Federation of April 1, 2016. No. 199n «On approval of the Rules of good laboratory Practice». The research protocol was approved by the Local Ethics Committee for conducting scientific research involving animals as research objects of the Lobachevsky State University on October 09, 2020.

CHF in rats was established by 3-fold (after 48 hours) intraperitoneal injection of epinephrine hydrochloride 0.3 mg/kg body weight. The dosage used causes metabolic damage to the myocardium with subsequent focal necrotization and corresponding circulatory disorders.

Totally 30 rats were randomly divided into the control group (n = 10), 1st research group (n = 10), 2nd research group (n = 10). Starting 1st day after inducting CHF, experimental animals of the 1st research group received forty-minute inhalation of 2% molecular hydrogen daily for 5 days, experimental animals of the 2nd research group received a single forty-minute inhalation of 2% molecular hydrogen and the control group did not receive hydrogen inhalation. The level of physiological norm was determined in intact animals. Blood samples were taken from the sublingual vein on 1 (for 1st and 2nd research – after molecular hydrogen inhalation), 3, 7, and 14 days after the CHF simulation. We estimated the intensity of LPO processes by the contents of diene (DC) and triene (TC) conjugates of polyunsaturated fatty acids (PUFAs), Schiff bases (SB) in blood plasma, which were determined by spectrophotometry on the RF-5301 PC spectrofluorometer, Shimadzu, (Japan). The malonic dialdehyde (MDA) concentration was determined by reaction with thiobarbituric acid to form a colored trimethine complex with maximum absorption at 530 nm. Catalase activity was analyzed according to the reduction of peroxide with the formation of water and oxygen (Deryugina et al., 2018).

Data obtained were processed using the application software packages Statistica 6.0 and Microsoft Excel (Microsoft, USA) using one-dimensional statistics methods. The results are presented as M±m, where M is the arithmetic mean, and m is the standard error of the mean. The significance of the differences in the averages was determined by the Student's t-test. The differences were considered significant at a significance level of $p < 0.05$.

Results

The interaction of free radicals with PUFAs which are esterified in membrane is the leading mechanism of cell damage under oxidative stress. Determination of the level of lipid peroxidation (LPO) products provides a comprehensive assessment of the severity of intoxication and processes of peroxidation.

The results showed the concentration of SB in the 1st and 2nd study groups was less than the level of the control group. No significant changes in the dynamics of DC and TC were detected (Fig. 1).

The lipid peroxidation process consists of three steps: initiation, propagation, and termination. In initiation, a diene conjugates (DC) formed as a result of separating of the allylic hydrogen from the arachidonic acid by prooxidants and subsequent stabilization by molecular rearrangement with double bond displacement. DC are toxic metabolites that have a damaging effect on lipoproteins, proteins, enzymes and nucleic acids. In the propagation phase, a lipid radical rapidly reacts with oxygen to form a lipid peroxy radical that abstracts hydrogen from another lipid molecule, generating a new lipid radical and lipid hydroperoxide (Saito et al., 2020). Secondary POL products are formed during the oxidative degradation of primary ones, including TC and carbonyl compounds, among which MDA. MDA is formed by decomposition of arachidonic acid and PUFAs, through enzymatic or nonenzymatic processes, and most often is measured for medical purposes. However, the primary and secondary ones are extremely unstable; in the presence

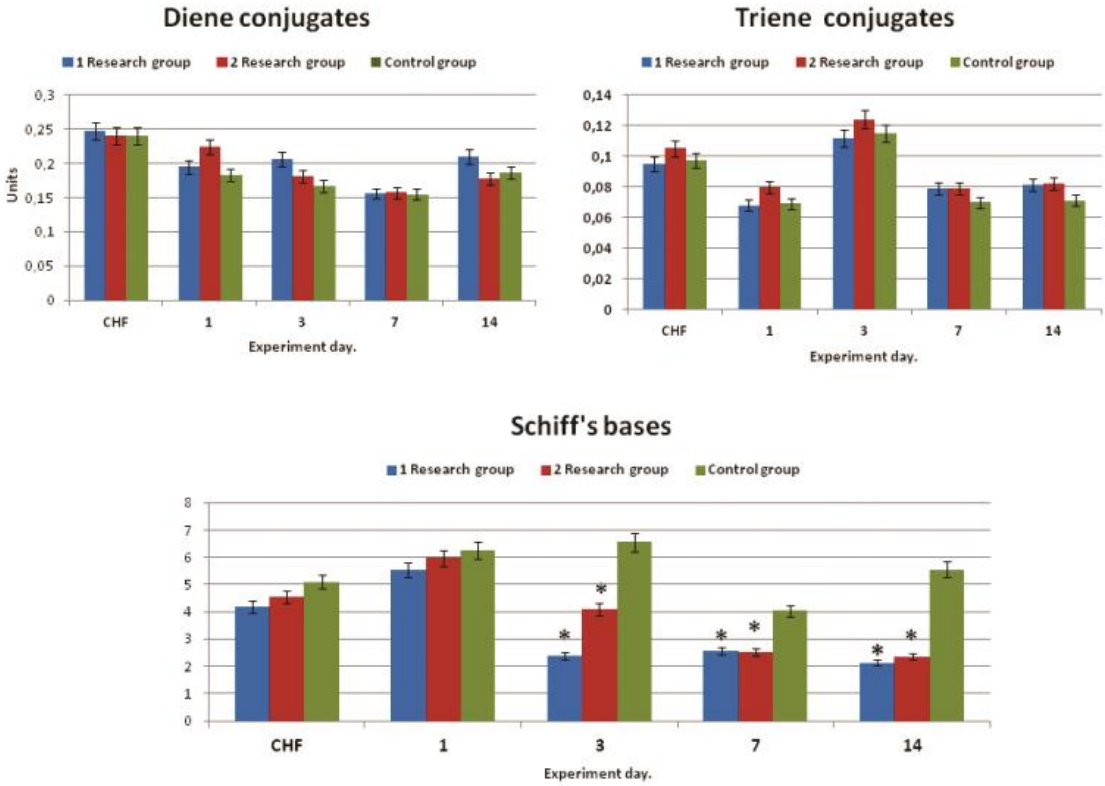

Fig. 1. Dynamics of diene and triene conjugates, and Schiff's bases in the blood samples of the compared groups (M ± m).
Notes: * – statistically significant differences in researches group compared to the control group at the research stages ($p \leq 0,05$)

of variable valence metals, they are rapidly metabolized into unsaturated substances containing the 1-amino-3-imino group (R-N=CH–CH=CH–NH–R) referred to as advanced lipoxidation end-products (ALE) (Moldogazieva et al., 2019) which include Schiff bases. The continuous accumulation of Schiff bases is an unfavorable sign and demonstrates the active course of POL, since they are the most stable and aggressive and, if excessive, lead to the destabilization of the cell membrane, and contribute to the destruction/death of cells (Deryugina et. al., 2018).

The examination of the dynamics of the MDA concentration in red blood cells showed a decrease in the indicator throughout the study with repeated exposure to molecular hydrogen and immediately after exposure with a single use of molecular hydrogen, followed by an increase in the indicator (Fig. 2).

In the termination reaction, antioxidants donate a hydrogen atom to the lipid peroxyl radical species, resulting in the formation of nonradical products (Saito et al., 2020). Catalase belongs to the group of antioxidants that reduce the formation of free radicals and interrupt chain reactions. This is a highly active enzyme with a high concentration in red blood cells, which metabolizes hydrogen peroxide (H_2O_2) to form water and oxygen without much energy expenditure (Zotti et al., 2020), its change allows us to judge the activity of the course of lipid peroxidation. The study of levels catalase activity in the red blood cells of rats showed its significant increase in both research groups compared to the control group (Fig. 2).

Here we investigate the possible correlation between its activity and concentration of SB. We observed the increase in catalase activity was inversely associated with concentration of SB in 1st ($r = –0,738$) and 2nd ($r = –0,671$) research groups. By contrast, control group had a weak direct correlation ($r = 0, 06$).

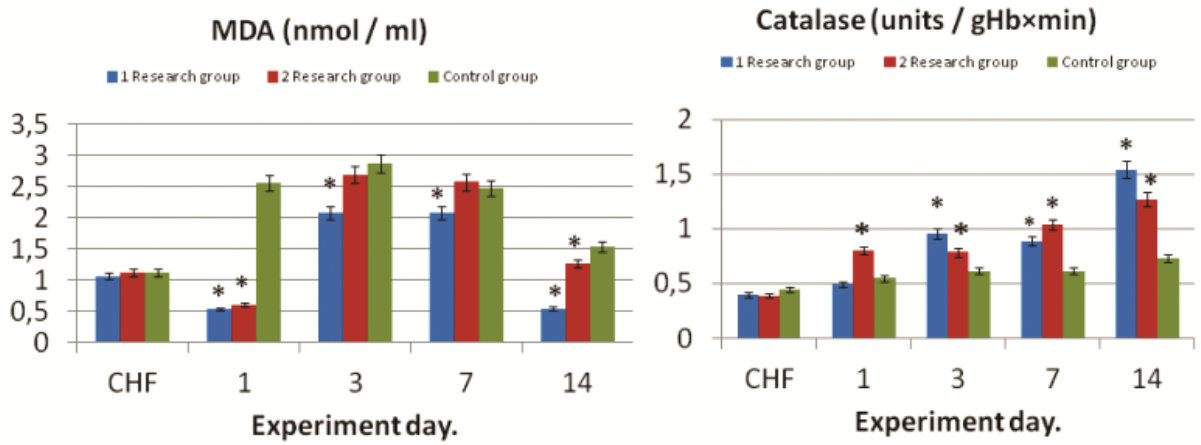

Fig. 2. Dynamics of MDA concentration and catalase activity in the compared groups (M ± m).
Notes: – statistically significant differences in researches group compared to the control group at the research stages ($p \leq 0,05$)

The relationship between catalase activity and concentration of MDA was also analyzed. The catalase activity levels showed a negative correlation (r = –0,133) with MDA in 1st research group. The catalase activity was weak positively correlated with MDA in 2nd research (r = 0,195) and control (r = 0,241) group, respectively.

Discussion

The complexly organized antioxidant defense is designed to maintain a delicate balance between the production of the physiological necessary level of ROS and the elimination of its excess amount, which leads to changes in metabolism, damage and cell death (Sies & Jones, 2020). Normally, the concentration of POL products is very low and is maintained at a relatively constant level. However, the accumulation of lipid peroxidation products occurs in a pathological situation leads to changes in the reaction of the lipid, hormonal, immune, and neurotransmitter statuses, the number of binding sites and affinity of receptors for ligands, depletion of the antioxidant system, and is the cause of diseases (Deryugina et al., 2020), including heart failure of various etiologies (Gaschler & Stockwell, 2017). At the same time, application of known antioxidants has limited therapeutic success, many clinically tested have demonstrated potential therapeutic possibilities, but none of them has shown unambiguous, pronounced efficacy.

Hydrogen, the smallest gas molecule with the highest diffusion capacity, can easily penetrate biological barriers (blood-brain, placental, and testicular barriers), and then enter the cytosol, mitochondria, endo/sarcoplasmic reticulum (ER), and nucleus via cell membranes. Therapeutic applications of H2 have been described in numerous animal experiments and clinical studies for diseases of various etiologies. Evidence suggests that H2 treatment confidently demonstrates antioxidant, anti-apoptotic and anti-inflammatory effects.

In 2007, Ohsawa et al. first demonstrated the ability of H2 to absorb hydroxyl radical (-OH) and peroxynitrite (ONOO-) was confirmed, even in the nuclear region of the cell, which is an important antioxidant property. Whereas the antioxidant enzyme for –OH, the most aggressive ROS, is still undetected, unlike O2 - superoxide dismutase (SOD) and H2O2 – peroxidase, the H2 administration makes it particularly promising in the treatment of diseases associated with oxidative stress.

In addition, in the presence of H2, there is an increase in the expression of cellular antioxidant (SOD, catalase, glutathione peroxidase, glutathione reductase, heme oxygenase-1 (HO-1) and others) (Hua et al., 2020). The data obtained in our study showed an increase in catalase activity in both research groups compared to the control group, which indicates high intracellular protection against ROS and, as a result, a decrease in oxidative stress.

Oxidative stress is the cause of an inflammatory tissue reaction. Recent reports also revealed that H2 downregulates pro-inflammatory and inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukins (IL-6, IL-1β) and macrophage chemoattractant protein 1, which could be mediated by suppressing the activation of nuclear factor kappa B (NF-kB), prostaglandin E2 (Qiu et al., 2019).

Considering the role of H2 in myocardial repair, it should be noted that ischemic/reperfusion (I/R) damage to the myocardium involves the closure of mitochondrial ATP-sensitive potassium channels (mitK-ATP), followed by the opening of mitochondrial pores (mPTP). An increase in the number of ROS leads to the release of calcium from the endoplasmic reticulum (ER) into the cytoplasm, which contributes to the destabilization of mitochondria, the opening of mitochondrial pores (mPTP), the release of cytochrome C (the final link of the electron transport chain) and apoptosis-inducing factor (AIF), acting as pro-apoptotic proteins (Colareda et al., 2016). H2 implements cardioprotective effects by promoting the opening of mitochondrial ATP-sensitive potassium channels (mitK-ATP) and the subsequent inhibition of mitochondrial permeability transition pores, which leads to an overall decrease in the mitochondrial membrane potential (Zhong et al. 2018; Barancik et al.2020).

The free diffusion of hydrogen molecules through tissues and cells suggests that H2 can act as a modulator of intracellular signaling, similar to the already known gas transmitters (nitrogen oxide (NO), hydrogen sulfide (H_2S), carbon monoxide (CO)). Recent data have documented multidirectional (both inhibitor and stimulator) effect of molecular hydrogen in the activation of distinct protein kinase cascades (Sano & Tamura, 2021). Recent reports also revealed that H2 contributes to the inhibition of endogenous apoptosis by acting on the enzyme glycogen synthase kinase 3β (GSK3ß) via PI3K/akt and the Wnt/β-catenin signaling pathway (wingless mouse-type mammary tumor virus family member integration/beta-Catenin) (Hu et al. 2020), the ROS-sensitive signaling pathways ERK1/2, p38, and JNK (Zhang et al. 2016), and also inhibited the activation of NF-kB by preventing the degradation of IkBa. In addition, H2 promotes inhibition of LPS/IFNy-induced NO production by modulating signal transduction in macrophages, thereby exhibiting an indirect anti-inflammatory effect.

Conclusions

Inhalation of H2 leads to a decrease in pro-oxidant and an increase in antioxidant parameters in red blood cells and blood plasma in rats with simulated chronic heart failure, which generally reduces the negative impact of POL and oxidative stress. The mechanisms of the cardioprotective effect of molecular hydrogen require further investigation.

Conflict of interest statement

Nothing declared.

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